The Characterization of Isoflavone Content in Soy Grits Before and After Its Incorporation Into Granola

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Background and Literature Review

Isoflavones are a type of phytochemical, a nonnutrient compound found in plants that is biologically active in the body. Isoflavones are almost exclusively found in legumes, especially soybeans. They occur mainly as glycosides (bound to a sugar molecule) in plants, but fermentation or digestion of soybeans or soy products results in the release of the sugar molecule from the isoflavone glycoside, leaving the isoflavone aglycones. Soybeans contain six major and six minor forms of isoflavones with genistin, daidzin, and glycitin being the most prevalent. The soybean cotyledon contains the majority of the ß-glycosides, genistin and daidzin and it also contains small proportions of the genistein and daidzein aglycone forms. The germ, on the other hand, contains high concentrations of glycitin and some glycitein, moderate amounts of daidzin, and relatively small amounts of genistin (1). Isoflavones are structurally similar to estrogen in that the phenol ring can bind to estrogen receptors on cells. Depending on concentrations and the specific tissues on which they act, isoflavones may exhibit agonistic or antagonistic estrogenic effects (by acting as estrogen mimics and imitating estrogen-like actions or by inhibiting estrogen actions) (2). Anti-estrogenic effects in reproductive tissue could help reduce the risk of hormone associated cancers (breast, uterine and prostate), while estrogenic effects in other tissues could help maintain bone density and improve blood lipid profiles (3). In fact, soy isoflavones have been marketed for use by women to help alleviate pre-menopausal symptoms and side effects of diminished natural estrogen in the body.

Genistein has been shown to inhibit tumor formation and proliferation. It affects cellular function via inhibition of 17β-steroid oxidoreductase (an enzyme necessary for estrogen synthesis) and tyrosine-specific protein kinases (4, 5). Isoflavones may reduce the risk of cancers (especially prostate and breast caners) as a result of their effects on apoptosis, cell cycle progression, cell growth and differentiation, as well as their antioxidant and antiangiogenic effects (5, 6).

Finally, the isoflavone content in soybeans may mediate a cholesterol lowering effect by affecting cellular sterol homeostasis, possibly via statin-like inhibition of HMG CoA reductase enzyme activity or by enhanced sterol regulatory element binding protein (SREBP) processing (7). It has been hypothesized that the maturation of SREBPs and induction of SRE-regulated genes produce an increase in surface LDL receptor expression, which causes an increase in the clearance of plasma cholesterol (7).

Research Questions

Because of the numerous possible health benefits of foods rich in soy, developing soy-based products that effectively deliver a high concentration of isoflavones and are satiably acceptable to the Western consumer is the motivation behind this research project. The purpose of the project is to develop a granola rich in soy isoflavones and to analyze and compare the isoflavone concentration in three types of soy grits before and after they are incorporated into granola. Previously, Lee et al found that there are significant differences in isoflavone content (including concentrations of aglycones, their glycoside conjugates, and total genistein, daidzein, and glycitein) among different soybean cultivars. The average total isoflavones in 17 Ohio soybean cultivars was 7.12

μmol/g soy. Total genistein content was the highest at 64.7% of total isoflavones, followed by total daidzein at 32.9% and total glycitein at 2.4% of total isoflavones (8). Based on the study by Lee et al, two Ohio soybean cultivars, Dwight and HF99-019, with a high total isoflavone concentration (10.602 and 11.753 μmol/g soy, respectively), and one soybean cultivar, HF01-0019, with a low total isoflavone concentration (4.317 μmol/g soy) were chosen for the soy granola analysis. The main purpose of this phase of the experiment is to investigate how granolaizing, soy grits affects isoflavone concentrations. Granolaizing is the process of mixing granola ingredients with a liquid binder that is liquid at elevated temperature (during baking), but sets when cooled to room temperature. Because of the elevated baking temperatures (ca. 325°F) and the mixing of soy grits with other ingredients (which may cause changes in pH and therefore affect isoflavone stability), it t is necessary to investigate how these processes affect the isoflavone profiles in the soy grit samples. Previous research has shown that there may be up to 65% loss in the total amount of isoflavones after processing compared to the initial concentrations in raw soybeans. Extraction procedures, temperature and pH effects on soy matrices, and/or the use of aqueous-alcohol solutions during the production of certain soy products (such as soy protein concentrates) may result in the decreased isoflavone concentration (9). Furthermore, Park et al, observed a 20.6% $\pm$ 7.9 SD decrease in total isoflavones for all soybean cultivars after heating them at 121°C (250°F) for 40 minutes (10).

In the final phase of the experiment, the three types of soy granola (with varying levels of isoflavones) and one oat-based granola (the standard) will undergo sensory testing in order to determine if the product is satiably acceptable to the consumer.

Materials and Methods

Materials: All 12 standard isoflavones, including daidzein, glycitein, genistein, daidzin, glycitin, genistin, malonyl daidzin, malonyl glycitin, malonyl genistin, acetyl daidzin, acetyl glycitin, and acetyl genistin, were purchased from LC Laboratories (Woburn, MA). DPPH, butylated hydroxytoluene (BHT), and formononetin were purchased from Aldrich (St. Louis, MO). High-performance liquid chromatography (HPLC)-grade methanol, acetonitrile, HCl, and acetic acid were purchased from Fisher Scientific (Fairlawn, NJ). Triplicate samples of each of the three raw soy grit samples and baked granola samples underwent extraction to isolate the isoflavones, followed by HPLC in order to analyze the isoflavone content. All chemicals used were of analytical grade or superior.

Extraction of soy isoflavones: 1.0-1.5 g of the homogenized sample was transferred to a 15 ml centrifuge vial and weighed. 0.3 μmol of 2',4' dihydroxy-2-phenylacetophenone (internal standard) was added to the vials, followed by 10 ml acetonitrile, 0.2 ml 1mol/L HCl, and 1.5 ml of distilled water. The samples were rigorously vortexed for 2 min. each, sonicated for 5 minutes, and then shaken for 1 hour on a shaker. The denaturized proteins were then separated by centrifugation at about 1000 x g for 5 minutes at room temperature. The final volume of the centrifuge vial was noted down. 1.5 ml of the aliquot was removed, weighed into a 10 ml glass vial, and evaporated to dryness under nitrogen in a cool (30 ^{o}C) waterbath. The residue was dissolved in 1500 μl methanol:water 80:20 and transferred into an HPLC vial. All samples were stored at -80^{o}C until HPLC analysis.

HPLC: A Hydrobond RP-18 HPLC column (100x3.0 mm, 3µm particle size, MacMod) and gradient elution was used: The mobile phase consisted of 1% acetic acid in water (solvent A) and 100% acetonitrile (solvent B) at a flow rate of 0.55 mL/min. The sample injection volume was 10 µL, and components were eluted using the following solvent gradient. from 0 to 2 min, solvent A was 85% and solvent B was 5%. From 2 to 20 min, the mobile phase was changed to 65%A and 35% B. From 20 to 23 min, solvent A was 25% and solvent B was 75%. From 23 to 29 min, solvent A was 85% solvent B was 5%. The temperature was 30°C.

The spectra were collected between 240 and 400 nm by PDA, and compounds in the eluate were detected at 260 nm.

Preparation of Granola: 127.4 g of soy grits and 6.1g of crisp rice were mixed and set aside. 52.0 g brown sugar, 27.6 g corn syrup solids, 8.2 g salt, 3.5 g baking soda, 2.7 g baking powder, 1.7 g soy lecithin granules, and 1.6 g cinnamon were mixed and set aside. 44.1 g honey, 10.7 g sunflower oil, 3.3 g apple juice concentrate, and 4.8 g orange juice concentrate were mixed and set aside. The Liquid and second mixture of dry ingredients were mixed and a small amount of water was added to thoroughly dissolve the brown sugar. This viscous mixture was then used to coat the soy grit mixture. The final mixture containing all ingredients was placed on a baking sheet and baked at 325°F until all water was evaporated (about 15 minutes).

Sensory Analysis: 30 students/faculty of The Ohio State University were recruited via email or in person to participate in the sensory analysis. Participants were over 18 years of age, non-pregnant, and not allergic to soy. Questionnaires were anonymous and the room was illuminated with red light so that the 4 granolas were not easily distinguishable. The three soy granolas and one oat-based granola (standard) were evaluated based on a hedonic scale, from extremely like to extremely dislike. Numbers were assigned to the rankings, with a score of 1 representing “extremely dislike” and a score of 9 represneting “extremely like”. One-way analysis of variance (ANOVA) was used to analyze the data in order to compare consumer acceptance of the four granola varieties.

Results and Discussion

Based on integration of the peaks of the chromatograms for the raw soy grits, the total isoflavone concentration in the cultivar “Dwight” was 2.5824 μmol/g sample. The total isoflavone concentration in the cultivar “HFI-0019” was 3.1271 μmol/g. The total isoflavone concentration in the cultivar “HF99-019 was 2.4942 μmol/g. The concentrations of the individual aglycone, ß-glycoside, acetyl-glycoside, and malonyl-glycoside forms for each of the three soy cultivars are depicted on table 1. The malonyl-glycosides comprised approximately two-thirds of the total isoflavone concentration, while the ß-glycosides comprised approximately one-third of the total isoflavone concentration. Only small quantities of the aglycone and aceteyl-glycosides were present in the samples.

The concentrations of the individual aglycone, ß-glycoside, acetyl-glycoside, and malonyl-glycoside forms for each of the three soy cultivars are depicted on table 2. Total isoflavone concentrations in the soy granola were present in significantly smaller amounts compared to the isoflavone concentrations in the soy grits. The total isoflavone concentration in the cultivar “Dwight” was 0.3479 μmol/g sample. The total isoflavone concentration in the cultivar “HFI-0019” was 0.4071 µmol/g. Finally, the total isoflavone concentration in the cultivar “HF99-019” was 0.4085 µmol/g. Compared to the data from the raw soy grits, the data for the soy granola suggests that approximately 13-16% of the total isoflavones present in the soy grits were also present in the soy granola. Because the recipe for the soy granola contained 43.4% soy grits, if no isoflavones were degraded due to processing effects, the expected isoflavone yields would be 43.4% less than the values for the soy grits. However, processing effects resulted in approximately 41-44% lower yields than what would be expected if processing did not affect the concentrations of the isoflavones. The several different processing steps involved in the conversion of the soy grits to the granola, including extractions, storage time, addition of other ingredients (which may have affected the pH), and elevated temperatures during baking may have resulted in the significant decrease in the total isoflavone concentrations.

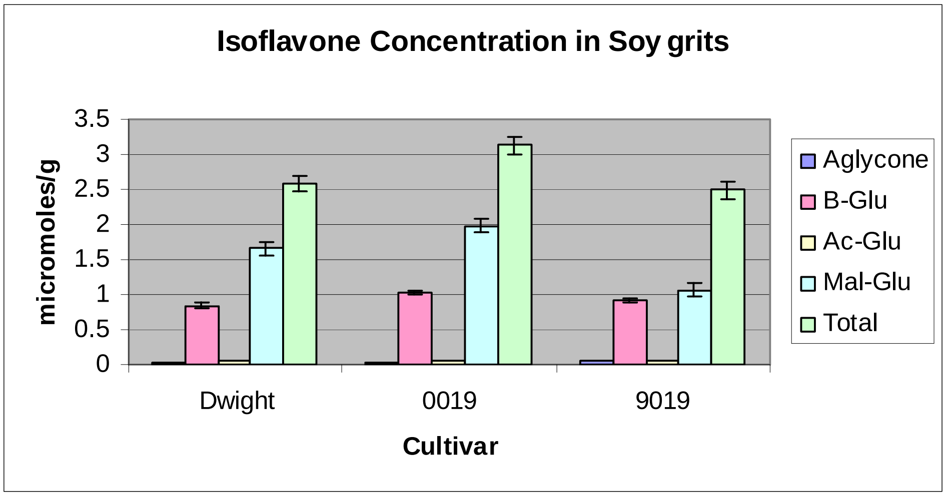
As depicted in graphs 1 and 2, the profiles for the families of the individual structures did not vary greatly between the soy grits and the granola. This suggests that the processing of the soy grits into granola did not greatly affect the conversion of one isoflavone to another within each family of isoflavones.

	Aglycone	B-Glu	Ac-Glu	Mal-Glu	Total
Dwight	0.03292	0.84586	0.04882	1.65487	2.5824
0019	0.04108	1.03016	0.04806	1.98358	3.12711
9019	0.04787	0.90667	0.04364	1.05980	2.49422

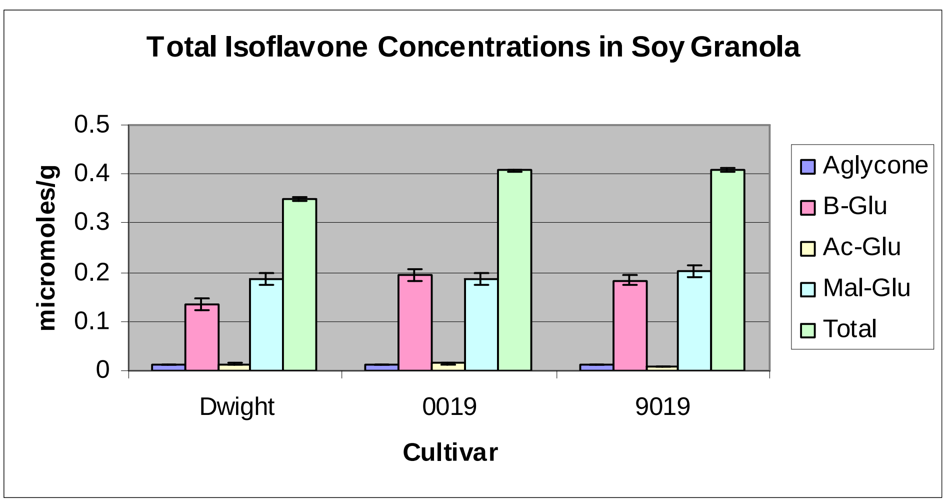
Table 1. Isoflavone content in raw soy grits (µmol/g)

	Aglycone	B-Glu	Ac-Glu	Mal-Glu	Total
Dwight	0.01249	0.13482	0.01373	0.18682	0.34786
0019	0.01117	0.19403	0.01444	0.18747	0.4071
9019	0.01281	0.18404	0.00835	0.20331	0.40851

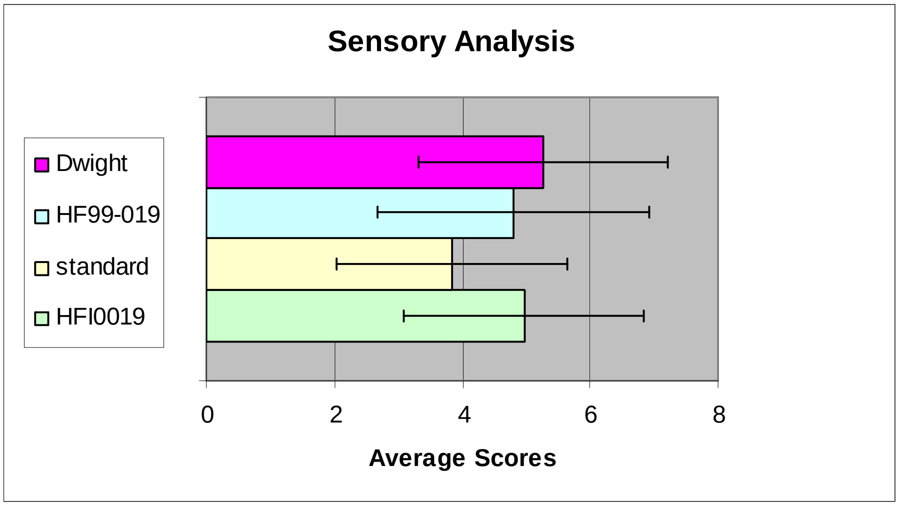
Table 2. Isoflavone content in soy granola (µmol/g)



Graph 1.



Graph 2.



Graph 3.

Graph 3 depicts the results from the sensory analysis. ANOVA revealed that significant variance exists (P=0.0319) between the consumer satisfaction results for the four granola samples; however, the three soy granola samples were favored over the standard oat-based sample. A few inferences can be made based on the sensory data. First, the formula for the granola could probably be improved since the average score for the standard oat-based sample was 3.83, which most closely correlates with "dislike slightly" on the hedonic scale. Secondly, based on the evidence that the soy granolas scored better than the oat granola, the soy grits could probably be incorporated into a granola that would be satiably acceptable to the Western consumer. Thirdly, the different soy granolas have different flavors, and the difference in flavors, according to ANOVA, is significant. It appears that the "Dwight" cultivar, which was the only granola to score above a rating of 5 (neither like nor dislike on the hedonic scale), has the best flavor.

In summary, it appears that soy grits can be incorporated into a granola that tastes comparable to a standard oat-based granola, but more research is needed to determine if the product would truly be satiably acceptable and marketable towards the Western consumer. The benefits of consuming such a product are also questionable since the isoflavone concentrations decreased significantly (41-44%) during processing. More research is needed in order to determine if the low yield of isoflavone concentrations in the granola were due to random or systematic errors, such as errors during extractions, degradation of isoflavones during storage (in -80ºC freezer), degradation of isoflavones in the presence of acid (required for extraction procedures), degradation of isoflavones due to the combining of the soy grits with other ingredients necessary to prepare the granola, or degradation of the isoflavones due to elevated temperatures during baking.

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